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IDENTIFICATION OF TYPES OF ECTOMYCORRHIZAE ON SEEDLINGS IN A BEECH PROVENANCE TRIAL

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Abstract
Root systems and types of ectomycorrhizae (ECM) were analysed on three 7-year-old beech seedlings (*Fagus sylvatica* L.) of three provenances (Val di Sella, Idrija, Nizbor) from an international beech provenance trial and on a naturally regenerated 5-year-old beech seedling from the same site (Kamenski vrh by Novo mesto). All short roots were mycorrhizal. In the sampled 20,302 root tips 49 % were non-turgescent and unidentifiable ECM. Twenty-two different types of ECM were identified, out of which 11 were successfully determined either to the species or genus level. All ECM were described by morphological and anatomical characteristics, basidiomycetes also by molecular methods. ECM that was not successfully determined either to the species or genus level formed a new cluster in the Slovenian mycorrhizal molecular database. Species richness and percentage of vital ECM roots were highest for the provenance from Idrija (Slovenia). The results indicate that there might be differences between provenances regarding the abundance of ECM, which should be further studied.

Key words: beech (*Fagus sylvatica* L.), provenance trial, types of ectomycorrhizae, PCR-RFLP

IDENTIFIKACIJA TIPOV EKTOMIKORIZE NA SADIKAH V BUKOVEM PROVENIENČNEM POSKUSU

Izvleček
V okviru mednarodnega bukovega provenienčnega poskusa na Kamenskem vrhu pri Novem mestu so bili v 3 ponovitvah analizirani koreninski sistemi in tipi ektomikorize (ECM) na 3 proveniencah (Val di Sella, Idrija, Nizbor) 7 letnih sadik bukve (*Fagus sylvatica* L.) in na 5 letnem vzorcu naravnega mladja. Skupno smo pregledali 20.302 korenini, od katerih je bilo 49 % starih, neturgescentnih ali nedoločljivih. Vse korenine so bile mikorizne. Ločili smo 22 različnih tipov ECM, od katerih smo 11 uspeli določiti do vrste ali rodu. Vse tipe smo opisali po morfološko-anatomskih kriterijih, bazidiomicete smo dodatno določevali z molekularnimi metodami. Neidentificirani tipi so tvorili v Slovenski mikorizni molekularni bazi podatkov nove grozde. Izračunani so bili indeksi raznovrstnosti. Največja diverziteta in delež vitalnih ektomikoriznih korenin sta bila ugotovljeni za provenienco iz Idrije. Pokazale so se manjše razlike med proveniencami glede prisotnosti ektomikorize, kar zahteva nadaljnje raziskave.

Ključne besede: bukev (*Fagus sylvatica* L.), provenienčni poskus, tipi ektomikorize, PCR-RFLP

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1 INTRODUCTION
UVOD

Mycorrhizal symbiosis is an association of fungi with roots acting as the chief organ of nutrient uptake by land plants, while fungi obtain organic carbon predominantly from the plant photosynthates (SMITH / READ 1997). Mycorrhizal mycelium as the principal link between different biotic and abiotic components in the forest soils is of utmost ecological importance for functioning of the forest ecosystems (KRAIGHER 1996, 2000). ECM increases the set of accessible and easily acquirable energy sources in the soil which should lead to biomass increase of the energy users (SETÄLÄ 2000) while the functional compatibility of the symbionts in mycorrhizae is species/strain and population specific (GIANNINAZZI-PEARSON 1984) and depends on physiological, anatomical and morphological characteristics of partners. Therefore mycorrhizae could contribute differently to a better growth of different provenances of forest trees.

In 1998 an international beech provenance trial was established in Slovenia to contribute to the estimation of genetically based suitability of different beech provenances for sustainable forest management in Europe (BOŽIČ *et al*. 2000). In order to asses the extent of genetic variation and to evaluate genetic resources it is necessary to know how different populations of the species are able to cope with different environments in both space and time (VON WUEHLISCH *et al*. 1997), which is accomplished by studying different traits in provenance trails. The adaptedness of a population to a given site is dependent on the population and the site conditions (MUHS 1997). Various site conditions have a strong influence on the expression of adaptive traits (*ibid*.).

We have studied types of ectomycorrhizae on three selected beech provenances and a naturally regenerated beech seedling in the beech provenance trial on Kamenski vrh nearby Novo mesto, thus contributing to the set of physiological parameters measured in the provenance trial. We presumed that if the same types of ectomycorrhizae appeared on all beech provenances, types of ectomycorrhizae might not be relevant for growth of different provenances.

2 MATERIAL AND METHODS
MATERIAL IN METODE

2.1 THE BEECH PROVENANCE TRIAL
BUKOV PROVENIENČNI POSKUS

The beech provenance trial was established with 31 beech provenances in 1998 in a mixed beech and silver fir stand classified as *Lamio orvalae-Fagetum* (HT.38)BORH.63 var. geogr. *Dentaria polyphyllos* KOŠ.62 (*Enneaphyllo-Fagetum* KOŠ.62 var. geogr. *Dentaria polyphylla* KOŠ.62) on the top of Kamenski vrh (N 45°47′46″/ E 15°02′54″, 544 m a.s.l.) (BOŽIČ *et al*. 2000). Soil type is classified as Eutric Cambisols and Haplic Luvisols. About 15 % of the surface is covered with rocks. The average annual precipitation for the period 1961 – 1990 is 1220 mm and the average yearly temperature is 9.5°C (EARS 2002).

Seedlings of all 31 provenances were grown at the Institute for Forest Genetics and Forest Tree Breeding in Grosshansdorf, Germany and imported to Slovenia as 2 year old seedlings in March 1998 (BOŽIČ *et al*. 2000). They were not tested for possible pre-colonisation with ECM originating from the nursery before planting. Since all seedlings were grown under the same conditions, this was not considered as an important factor.

2.2 SAMPLING
VZORČENJE

The provenance trial was established as a block trial in 3 blocks, each representing one repetition. In each block 31 provenances were randomly assigned to 31 plots. On each plot 50 seedlings were planted in 5 rows. Distance between rows was 2 m and between seedlings in a row 1 m. Root systems and types of ECM on 3 beech provenances from Val di Sella (Italy), Idrija (Slovenia) and Nizbor (Czech Rep.) (Table 1) were analysed in 3 repetitions. One seedling from each plot in each block was selected in places where seedlings were dense (as there was no previous loss of seedlings due to biotic and/or abiotic reasons) and where in the close future thinning would be needed. Additionally, a sample of naturally regenerated beech from the second block was analysed (10 samples in total). Whole root systems of 7 year old seedlings were taken from the soil (in May 2003) and stored at 4 ºC until processing.

2.3 ANALYSES OF THE ROOT SYSTEM
ANALIZE KORENINSKEGA SISTEMA

On the basis of the assumption that roots grow evenly in all directions, root systems still covered by soil were cut in half vertically. One half was randomly selected for analysis and left in water for one night to be rinsed the next day and the other half was stored for future analysis. The shape of the root system was assessed visually. The highest root in the root system was cut off for further analysis. Its length and upper diameter were measured (Table 1).

2.4 IDENTIFICATION OF ECTOMYCORRHIZAE
IDENTIFIKACIJA EKTOMIKORIZE

Types of ECM were analysed and identified after morphological and anatomical characteristics (AGERER 1987-2002, AGERER 1997-2002, AGERER 1991, BRAND 1991) with a binocular Olympus 12 SZX 12 0'5X and microscope Olympus DP 12 BX 51. Types of ECM were documented with Image Analysis® software and included into the *Mycotheca and Herbarium of the Slovenian Forestry Institute*. Additionally, Basidiomycetes were analysed by PCR- RFLP of the ITS region of rDNA. In average 2 to 3 root tips per morphotype were subjected to PCR-RFLP identification. DNA extraction, amplification and RFLP analysis were carried out as described in KRAIGHER *et al*. (1995) using a pair of primers ITS1f and ITS4b (GARDES / BRUNS 1993) and restriction endonucleases *Hinf* I, *Mbo* I and *Taq* I (KÅREN *et al*. 1997, GREBENC *et al*. 2000). RFLP patterns were analysed with Taxotron® software (GRIMONT 1998). The results were compared with the RFLP patterns from the Slovenian Forestry Institute database of RFLP patterns (GREBENC / KRAIGHER 2000 – extended) using Unweighted Pair Group Method of Averages and method Single Linkage.

2.5 DIVERSITY INDICES
INDEKSI RAZNOVRSTNOSTI

Species richness (d), Shannon – Weaver Index of Diversity (H) and Equitability (J) were calculated after ATLAS and BARTHA (1981).

3 RESULTS
REZULTATI

In 4 cases root systems were growing only in one direction presumably because of the rocky soil surface. The average length of the analysed long roots was 19.1 cm and their diameter varied from 3.0 to 7.5 cm. Short mycorrhizal roots were both unbranched and branched.

Table 1: Elevation of the basic material, time of flushing, seedling height, average length and broadest diameter of analysed roots (as by May 2003)
Preglednica 1: Nadmorska višina izhodiščnega materiala, čas olistanja, višina, povprečna dolžina in največji premer analiziranih korenin sadik bukve (stanje maja 2003)

Provenance / Provenienca	Elevation / Nadm. višina [m]	Flushing / Olistanje	Height / Višina [cm]	Length / Dolžina [cm]	Diameter / Premer [cm]
Val di Sella (Italy)	1150	early / zgodnje	159	17,5	6,3
Idrija (Slovenia)	930	late / pozno	112	16,5	3,7
Nizbor (Czech Republic)	480	medium / srednje	100	18,4	4,7

All short roots were mycorrhizal. In 20,302 root tips no non-mycorrhizal roots were found. Forty-nine percent of ECM root tips were old, non-turgescent or unidentifiable. The highest percentage of old, non-turgescent or unidentifiable root tips was observed in the provenance from Italy and the highest percentage of vital ECM root tips on the provenance from Slovenia (Figure 1).

Twenty-two different types of ECM were identified out of which 11 were successfully determined either to the species or the genus level. These types were *Laccaria amethystina* (Bolt. ex Hooker) Murr., *Russula cyanoxantha* (Schaeff.) Fr., *Russula illota* Romagn., *Lactarius pallidus* Pers. ex Fr., *Cenococcum geophylum* Fr., *Fagirhiza setifera*, *Fagirhiza fusca*, *Cortinarius* sp. 1, *Cortinarius* sp. 2, *Cortinarius* sp. 3 and *Russula* sp. Ten types of ECM (SLOMP01 – SLOMP10) had not been identified previously. Additionally, one out of 3 mycorrhizal root tips of the unidentified type SLOMP05 subjected to PCR-RFLP analysis gave a different RFLP pattern. We could not find any differences with the morphological-anatomical method; therefore, we referenced it as a SLOMP11. The overall PCR amplification rate was 77 %.

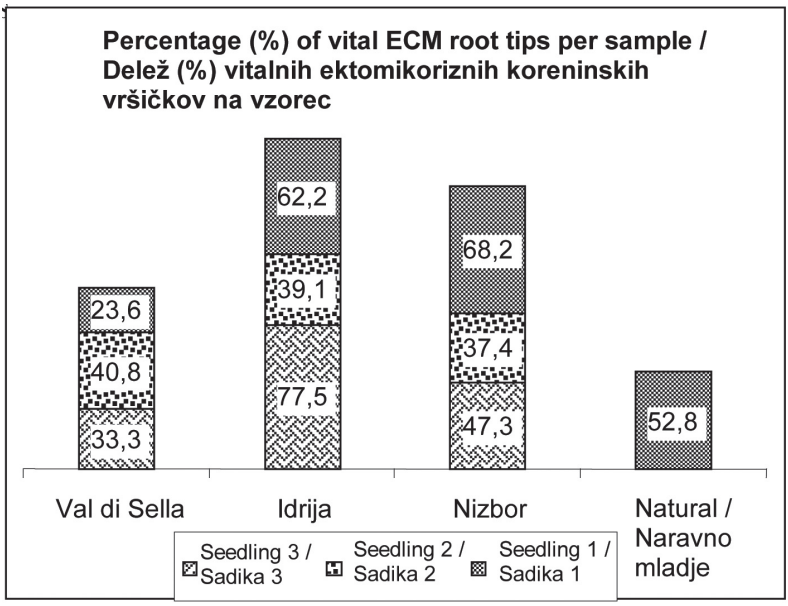


Figure 1: Percentage of vital ECM root tips per sample
Slika 1: Delež vitalnih ektomikoriznih koreninskih vršičkov na vzorec

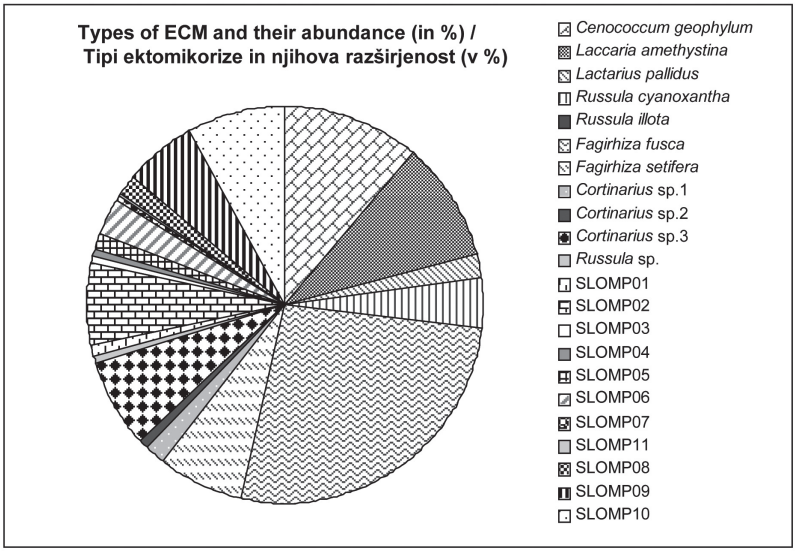


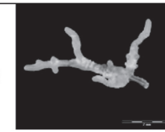
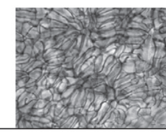
Figure 2: Abundance of vital ECM root tips per type of ECM for all 3 provenances and a sample of naturally regenerated beech
Slika 2: Pogostnost vitalnih ektomikoriznih koreninskih vršičkov za 3 provenience in vzorec naravnega mladja

3.1 DESCRIPTION OF UNIDENTIFIED TYPES OF ECM
OPIS NEIDENTIFICIRANIH TIPOV EKTOMIKORIZE

Table 2: Description of unidentified types of ECM and types identified to genus level
Tabela 2: Opis neidentificiranih tipov ECM in tipov ECM določenih do rodu

Type ID / Oznaka tipa ECM	Ramification & growth/ Razrast & oblika rasti	Colour, mantle surface / Barva, površina plašča	Emanating elements / Izhajajoči elementi	Mantle anatomy (after Agerer, 1987-2002) / Anatomija plašča (po Agerer, 1987-2002)	Anatomy of rhizomorphs / Anatomija rizomorfov	Length of ECM [mm] / Dolžina ECM [mm]	Width of ECM [mm]/ Širina ECM [mm]	Other / Drugo
Cortinarius sp. 1	irregularly pinnate, bent	white to yellow, smooth to woolly	numerous bright hyphae, rizomorphs	plectenhymatous	slightly differentiated	1,00–4,50	0,25	
Cortinarius sp. 2	irregularly pinnate, tortuous	brown with silver shine, silvery	white rizomorphs	plectenhymatous (type B)	mostly undifferentiated	0,80–7,00	0,25–0,30	anastomoses present
Cortinarius sp. 3	irregularly pinnate, slightly tortuous	yellow to brown, smooth to woolly	bright hyphae	partially cowered with soil, plectenhymatous with long thin hyphae arranged parallelly	not observed	1,50–15,00	0,25–0,35	
Russula sp.	monopodial pinnate, straight	white, covered with soil particles	hyphae septated, surrounded with gelatinous cover	pseudoparenchymatous with angular cells	not observed	1,00–4,75	0,25–0,35	
SLOMP01	monopodial pinnate, straight	white, grainy	not observed	plectenhymatous with no distinguishable pattern, in lower levels pseudoparenchymatous with angular cells	not observed	1,35–3,00	0,20–0,25	when touched or injured, the colour changes into grey
SLOMP02	monopodial pinnate, bent	yellow-brownish, smooth	infrequent hyphae, without clamps	plectenhymatous (type B)	not observed	0,50–3,50	0,15–0,20	
SLOMP03	monopodial pinnate, straight	white, smooth	infrequent white hyphae, short with simple septa, without clamps	plectenhymatous	not observed	0,50–3,00	0,25–0,35	Sulfo-vanillin: negative 
SLOMP04	monopodial pinnate, straight	white, grey in the basis, smooth	infrequent bright hyphae, with clamps	plectenhymatous with strongly branched hyphae distributed like a net	not observed	0,25–1,50	0,15–0,25	

Table 2: Description of unidentified types of ECM and types identified to genus level (continuation)
Tabela 2: Opis neidentificiranih tipov ECM in tipov ECM določenih do rodu (nadaljevanje)

SLOMP05	irregularly pinnate, bent	white, smooth	infrequent hyphae, septated, without clamps	pseudoparenchymatous with oval, sometimes pointy cells	not observed	0,50–3,10	0,30–0,40	Sulfo-vanillin: some cells gave positive reaction	
SLOMP06	monopodial pinnate, bent	brown, grainy	infrequent hyphae with simple septa	partially cowered with soil parts, pseudoparenchymatous with angular cells	not observed	1,00–6,00	0,40–0,50		
SLOMP07	monopodial pinnate, straight with bent apexes	white, grainy	not observed	pseudoparenchymatous with angular cells	not observed	0,75–3,00	0,25		
SLOMP08	monopodial pinnate, straight	white, partially yellowish, grainy	infrequent hyphae	plectenhymatous with gelatine matrix among hyphae (type C)	not observed	0,50–3,50	0,25–0,35	rare cystidia	
SLOMP09	monopodial pinnate, straight to slightly bent	yellow to brown, smooth	short bright hyphae with clamps, denser at the base of the tip	plectenhymatous without noticeable grouping pattern (type B – C)	not observed	0,60–5,75	0,20–0,25	soil sticking to the mantle at the base of the tip	
SLOMP10	monopodial pinnate, straight to bent	dark brown to black, grainy	infrequent black hyphae with clamps, black rizomorphs growing rectangularly in the mantle	plectenhymatous, hyphae forming a star pattern	undifferentiated	0,50–8,00	0,20–0,25	KOH - negative	

Species richness for samples varied between 0.71 and 1.71 (Table 3). The lowest Equitability (0.44) was found in the sample Idrija-3, where *Fagirhiza fusca* accounted for 78 % of all vital ECM root tips.

Table 3: Species richness (d), Shannon-Weaver Index of Diversity (H) and Equitability (J) per sample
Preglednica 3: Bogastvo vrst (d), Shannon-Weaverjev indeks diverzitete (H) in izenačenost (J)

Provenance and sample number / Provenienca in številka vzorca	Diversity indices / Indeksi pestrosti		
	d	H	J
Val di Sella-1	0.71	0.86	0.78
Val di Sella-2	0.86	0.65	0.60
Val di Sella-3	1.18	1.21	0.88
Idrija-1	1.71	1.54	0.86
Idrija-2	1.36	0.97	0.61
Idrija-3	1.46	0.79	0.44
Nizbor-1	1.23	0.92	0.57
Nizbor-2	1.11	0.81	0.59
Nizbor-3	0.88	0.87	0.80
Natural	1.48	1.40	0.78

Table 4: Number of types of ECM, number of vital root tips, number of old, non-turgescent or unidentifiable root tips and diversity indices for the three beech provenances
Preglednica 4: Število tipov ECM, število vitalnih koreninskih vršičkov, število starih, neturgescentnih ali nedoločljivih tipov in indeksi pestrosti za tri bukove provenience

Characteristic / Značilnost	Provenance / Provenienca		
	Val di Sella	Idrija	Nizbor
Number of types / Število tipov	8	14	9
Number of vital ECM / Število vitalnih ektomikoriz	1213	4338	2522
Number of old, non-turgescent, unidentifiable ECM / Število starih, neturgescentnih ali nedoločljivih ektomikoriz	3127	2718	1916
Species richness (d) / Indeks bogastva vrst	2.27	3.57	2.35
Shannon–Weaver Index (H) / Shannon-Weaverjev indeks pestrosti (H)	1.80	1.68	1.59
Equitability (J) / Indeks izenačenosti (J)	0.87	0.64	0.72

Only *Cenococcum geophylum* was found in all 10 samples. On 3 provenances (but not on the sample from naturally regenerated beech) *Lactarius pallidus* type was found. Six types of ECM were identified on 2 provenances and 13 types were found on only 1 provenance or on the sample from naturally regenerated beech. Six out of these 13 types of ECM were found on the provenance from Idrija (Table 5).

Table 5: Percentage of vital ECM root tips per provenance and the sample of naturally regenerated beech
Preglednica 5: Delež vitalnih koreninskih vršičkov za provenience in vzorec naravnega mladja

	Val di Sella	Idrija	Nizbor	Natural	% vital ECM
Cenococcum geophylum	1.6	8.1	1.3	0.2	11.2
Laccaria amethystina	0.0	0.1	9.5	0.0	9.5
Lactarius pallidus	1.4	0.7	0.1	0.0	2.2
Russula cyanoxantha	0.0	0.8	3.1	0.0	3.9
Russula illota	0.0	0.2	0.0	0.0	0.2
Fagirhiza fusca	0.0	20.0	1.5	5.1	26.6
Fagirhiza setifera	0.0	0.0	6.9	0.0	6.9
Cortinarius sp.1	1.5	0.0	0.1	0.0	1.6
Cortinarius sp.2	0.0	0.0	1.1	0.0	1.1
Cortinarius sp.3	0.0	3.1	0.6	3.3	7.0
Russula sp.	0.3	0.2	0.0	0.1	0.6
SLOMP01	0.0	0.9	0.0	0.0	0.9
SLOMP02	4.1	2.5	0.0	0.0	6.7
SLOMP03	0.4	0.0	0.0	0.0	0.4
SLOMP04	0.5	0.0	0.0	0.0	0.5
SLOMP05	0.0	1.5	0.0	0.0	1.5
SLOMP06	0.0	3.0	0.0	0.0	3.0
SLOMP07	0.0	0.2	0.0	0.0	0.2
SLOMP08	1.7	0.0	0.0	0.0	1.7
SLOMP09	0.0	0.0	0.0	5.9	5.9
SLOMP10	0.0	0.0	0.0	8.0	8.0
SLOMP11	0.0	0.2	0.0	0.0	0.2

Fagirhiza fusca accounted for 27 %, *Cenococcum geophylum* for 11 % and *Laccaria amethystina* for 10 % of all vital ECM root tips. All other types were present in percentages lower than 10% of all vital ECM root tips (Figure 2, Table 5).

4 DISCUSSION
DISKUSIJA

In our study we have combined the basic physiological studies of the beech provenance trial, especially growth characteristics and phenology, with studies of types of ectomycorrhizae. This is to our knowledge the first such approach in beech provenance trials in Europe. For identification of ECM we have combined the morphological-anatomical method with the molecular method as developed at the Slovenian Forestry Institute and its PCR-ITS-RFLP database. The overall PCR amplification rate was 77 %. We presume this is a consequence of the choice of the primers used for amplification, since the primer pair ITS1f and ITS4b only amplifies basidiomycetes. Additionally, it is known that old ECM is hard to amplify (GARDES / BRUNS 1993, GREBENC / KRAIGHER 2000).

The highest percentage of old, non-turgescent or unidentifiable types of ECM and the lowest species richness (d) were found on the provenance from Italy, which has the highest average height (159 cm) and biggest diameter of analysed roots. This supports results by BAXTER and DIGHTON (2001) showing that inoculation with higher number of ECM fungi resulted in lower biomass of aboveground part of the seedling and increased mass of the roots; the total biomass in their pot trial did not change with respect to the number of species and their combination in inoculum.

The highest diversity (Table 4), percentage of vital ECM root tips (Figure 1) and percentage of types of ECM which appear only in one of the provenances was found on the provenance from Idrija (Slovenia) which seems to be best adapted to conditions in which the provenance trial was established. The diversity of ECM increases the efficiency of nutrient uptake from different soil types (LEAKE 2001). It is possible that the high diversity of ECM is responsible for the relatively big height increment despite the short vegetation period of the late flushing Idrija provenance.

The beech provenance trial was established on a clear-cut in a mixed beech and silver fir stand classified as *Lamio orvalae-Fagetum* (HT.38)BORH.63 var. geogr. *Dentaria polyphyllos* KOŠ.62. Despite the removal of the plant partner, the ECM mycelium is still present in the soil in smaller gaps and represents one of the potential sources of inoculation of germinating seeds or planted seedlings (AGERER 1987-2002, SMITH / READ 1997). Diversity of ECM fungi and statistically significant differences in species composition show a negative influence on the community of ECM fungi and the whole ecosystem immediately after a clear-cut (BYRD *et al*. 2000, KRAIGHER 1999). However, diversity indices in our study (Species richness, Shannon–Weaver Index of Diversity and Equitability) were high.

Shannon–Weaver Index of Diversity per provenance varied between 1.59 and 1.80 (Table 3) and was comparable with the same index found on polluted research plots in beech forest stands in Zavodnje and Dobovec, where its value was 1.8 (AL SAYEGH PETKOVŠEK / KRAIGHER 2004). The value for two unpolluted plots was 1.9 *(ibid.*). Species Richness and Equitability were also comparable to the results of the above mentioned study, d was 3.1 (2.9) and J was 0.7 on polluted and unpolluted plots.

It is known that after a perturbation new species of ECM can appear as colonisers (BRUNNER 2001, ERLAND / TAYLOR 2002). Therefore after an initial decrease of diversity, the early succession ECM might appear on a replanted site. Our study was done 5 years after replanting, thus allowing different succession phase fungi to appear on the site, colonizing the different provenances according to their physiological state, to the different closure of canopies of the seedlings, planted in rows and the quality of their litter. This can explain the high species diversity in total as well as differences among ECM on different provenances.

Our research took part in a former clear-cut. Types of ECM that were not successfully determined either to the species or genus level formed 4 new clusters in the Slovenian Forestry Institute PCR-ITS-RFLP database. The database consists of RFLP patterns of sporocarps and types of ECM from mature managed beech, beech-silver fir and beech-spruce forests and forest reserves. We conclude that types of ECM found on beech seedlings in the provenance trial differ from types of ECM found in mature forests. For identification of unidentified types of ECM sporocarps would have to be collected in the area of the provenance trial, subjected to PCR-RFLP analysis and compared to RFLP patterns obtained from ECM root tips.

Four black types of ECM were identified in samples: the ascomycete *Cenococcum geophylum*, basidiomycetes *Fagirhiza fusca*, *Fagirhiza setifera* and an unidentified basidiomycete SLOMP10. PCR-RFLP analysis showed that the latter 3 types belong to 3 different species, which formed a new cluster in the RFLP patterns database. Therefore we assume that they are likely to belong to the same genus of Basidiomycetes. Type SLOMP10 had rhizomorphs and showed similarities to *Tomentella ferruginea* (Pers.) Pat. ECM, but parts of the mantle and rhizomorphs did not stain greenish to bluish in KOH, which is typical for ECM formed by *Tomentella ferruginea*. Another difference was the lack of peripheral ensheating hyphae growing in a twisted manner covering the inner hyphae of rhizomorphs.

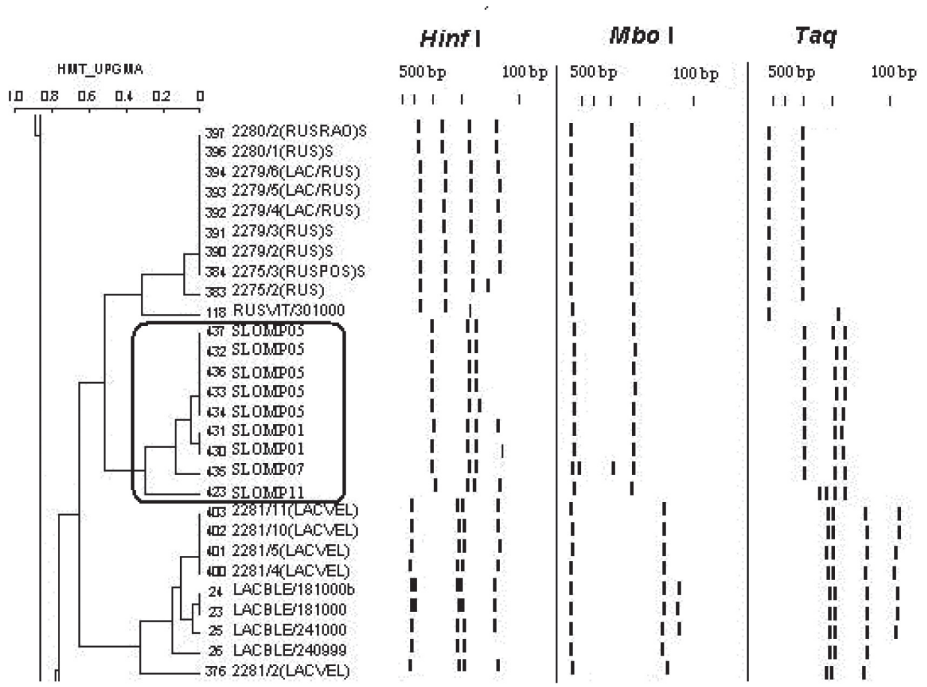


Figure 3: Part of the PCR-ITS-RFLP dendrogram of the Slovenian mycorrhizal molecular database – extended with a new cluster
Slika 3: Del PCR-ITS-RFLP dendrograma Slovenske mikorizne molekularne baze podatkov, razširjenega z novim grozdom

The primary succession of the site is accompanied with the succession of the ECM fungi (LAST *et al*. 1987). JUMPPONEN *et al*. (2002) have described a number of ECM fungi on different sites in different phases of primary succession. Due to the thinning on the plot in the near future, this study could be a beginning of a long term monitoring of ECM succession on beech without any negative impact on the provenance trial.

5 POVZETEK

Mikorizni micelij predstavlja pomemben povezovalni element med biotskimi in abiotskimi dejavniki v gozdnih tleh. Pomen mikorize za rastline se lahko kaže v boljšem razvoju in rasti rastlinskega partnerja v primerjavi s tistim, ki ni mikorizen. Funkcionalna kompatibilnost simbionta v mikorizi je vrstno oziroma populacijsko specifična in

odvisna od fizioloških, anatomskih in morfoloških lastnosti partnerja. Mikoriza bi zato lahko prispevala k boljši rasti posameznih provenienc. Analizirali smo tipe ECM, ki se pojavljajo na 3 proveniencah bukve (Val di Sella, Idrija, Nizbor) v okviru mednarodnega bukovega provenienčnega poskusa na Kamenskem vrhu pri Novem mestu ter na vzorcu naravnega mladja. Z raziskavo smo prispevali k setu fizioloških parametrov, merjenih v provenienčnem poskusu. V primeru, da bi se na vseh proveniencah pojavljali isti tipi ektomikorize (ECM), bi lahko sklepali, da le-ti ne vplivajo na razlike v rasti med različnimi proveniencami.

Za analizo, ki smo jo opravili v 3 ponovitvah, smo izbirali korenine, ki so iz koreninskega vratu izraščale najvišje in so bile približno enakih dimenzij. Opisali smo tudi koreninski sistem, ki je bil v polovici primerov lepo razvit in simetričen, v polovici pa zavit in je rastel v eno smer.

Tipe ECM smo določevali po morfološko-anatomski metodi in s PCR-RFLP ITS regije v rDNK z začetnima oligonukleotidoma ITS1f in ITS4b.

Pestrost tipov ECM na območju provenienčnega poskusa je velika. V 10 vzorcih smo analizirali 20.302 mikorizni korenini, od katerih je bilo 49 % starih, neturgescentnih oziroma nedoločljivih mikoriznih korenin, in določili 22 tipov ECM, od katerih smo do vrste ali rodu uspeli določiti 11 tipov (*Laccaria amethystina*, *Russula cyanoxantha*, *Russula illota*, *Lactarius pallidus*, *Cenococcum geophylum*, *Fagirhiza setifera*, *Fagirhiza fusca*, 3 tipi *Cortinarius* sp., *Russula* sp.). Primerjava RFLP vzorcev tipov ECM, najdenih na različnih proveniencah, s Slovensko mikorizno molekularno bazo podatkov ni dala zadovoljivih rezultatov, saj je večina tipov ECM s provenienc tvorila nove grozde v dendrogramu. Predvidevamo, da so tipi ECM, ki se pojavljajo na sadikah bukve iz provenienčnega poskusa, različni od tipov, ki se pojavljajo v odraslem gozdu.

Fagirhiza fusca predstavlja 27 %, *Cenococcum geophylum* 11 % in *Laccaria amethystina* 10 % vseh vitalnih ECM korenin. Preostali tipi so prisotni z deležem, manjšim od 10 % (Slika 2). V vseh vzorcih smo našli le *Cenococcum geophylum*. Na vseh 3 proveniencah, ne pa tudi v vzorcu naravnega mladja, se pojavlja *Lactarius pallidus*. Na 2 proveniencah se pojavlja 6 tipov ECM. Na 1 provenienci ali na naravnem mladju se pojavlja 13 tipov, 6 izmed njih najdemo le na provenienci iz Idrije. Velik delež tipov, ki se v večjem ali manjšem številu pojavljajo le na eni provenienci ali na naravnem mladju, daje slutiti, da med proveniencami obstajajo razlike.

Skupni indeks bogastva vrst (d) znaša 5,23, d za provenienco iz Idrije znaša 3,57, za provenienco iz Italije 2,27 in za provenienco iz Češke 2,35. Indeks bogastva vrst za posamezne vzorce se giblje med 0,71 in 1,71. Shannon-Weaverjev indeks pestrosti se giblje v intervalu med 1,59 in 1,80 in je primerljiv z istim indeksom, izračunanim za vzorce, nabrane na onesnaženih (Zavodnje, Dobovec) in neonesnaženih bukovih raziskovalnih ploskvah v Sloveniji (AL SAYEGH PETKOVŠEK / KRAIGHER 2004). Tudi indeks izenačenosti (J), ki je neodvisen od velikosti vzorca in pokaže, ali obstajajo v vzorcu dominantne populacije, se giblje med 0,44 in 0,88 in je primerljiv z rezultati omenjene študije.

Če upoštevamo kazalca delež vitalnih mikoriznih korenin in bogastvo vrst, lahko sklepamo, da je provenienca iz Idrije bolj prilagojena rastišču in razmeram, v katerih je osnovan provenienčni poskus, kot pa provenienci iz Italije in Češke. Tudi delež tipov ECM, ki se pojavljajo le na eni provenienci, je za provenienco iz Idrije največji.

Da bi lahko tipe ECM, ki jih v bazi podatkov še ni, določili do vrste, je potrebno nabiranje trosnjakov na območju provenienčnega poskusa, njihova PCR-RFLP analiza in primerjava njihovih RFLP vzorcev z RFLP vzorci najdenih tipov ECM.

Z analizo večjega števila vzorcev za posamezne provenience bomo našli odgovor na vprašanje, ali med tipi ECM, ki se pojavljajo na različnih proveniencah, obstajajo statistično značilne razlike in če obstajajo, ali so pomembne za uspevanje posameznih provenienc.

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